**2910-Pos Board B680**
**Voltage Dependent Anion Channel-3 (VDAC3) is the Major Isoform Contributing to Mitochondrial Metabolism in HepG2 Cells and is Regulated by Free Tubulin and Erastin**
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**BACKGROUND:** VDAC controls the flux of hydrophilic metabolites into mitochondria. *In vitro,* tubulin closes VDAC and *in situ* free tubulin dynamically modulates $\Delta\Psi$. Erastin, a ligand for VDAC, increases $\Delta\Psi$ in HepG2 cells. Here, we hypothesize that VDAC regulates mitochondrial metabolism and that free tubulin decreases and erastin increases VDAC conductance. Our **AIM** was to determine $\Delta\Psi$, ATP, NADH, and response to free tubulin and erastin after VDAC knockdown (KD). **METHODS:** HepG2 cells were transfected with siRNAs (5 nM, Ambion) against VDAC1/2/3. At 48 h after transfection, $\Delta\Psi$ was assessed by fluorescence of tetramethylrhodamine methylester (TMRM) and NADH by autofluorescence using confocal/multiphoton microscopy. Fluorescent beads were fiduciary markers. Adenine nucleotides were determined by HPLC. **RESULTS:** siRNA decreased mRNA and protein for each VDAC isoform by ~90%. Double KD of VDAC1/2, VDAC1/3 and VDAC2/3 decreased TMRM fluorescence by ~20, 55 and 73%, respectively, compared to 100% for non-target siRNA. In reconstituted bilayers, VDAC from HepG2 formed typical anion selective and voltage-gated channels reversibly blocked by dimeric tubulin. Nocodazole decreased $\Delta\Psi$ by ~61% in non-target cells and 43, 14 and 17% after KD of VDAC1/2, VDAC1/3 and VDAC2/3. VDAC3 KD decreased ATP by ~48%, total adenine nucleotides by ~45%, NADH by ~33% and the NADH/NAD ratio by ~60%. Erastin increased $\Delta\Psi$ by ~46-48% in non-target and VDAC1/2 KD cells but failed to return $\Delta\Psi$ to baseline levels after VDAC1/3 and VDAC2/3 KD. Erastin also blocked and reversed depolarization induced by nocodazole. **CONCLUSION:** VDAC3, the least abundant isoform, contributes most to maintenance of mitochondrial metabolism in HepG2 cells. Erastin antagonizes the inhibitory effect of free tubulin. These results indicate that VDAC, especially VDAC3, regulates mitochondrial metabolism in hepatoma cells.

**2911-Pos Board B681**
**Nrf2 Controls Mitochondrial Bioenergetics**
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The transcription factor nuclear factor E2-related factor 2 (Nrf2) and its repressor Kelch-like ECH-associated protein 1 (Keap1) are known to regulate the antioxidant response element (ARE) pathway responsible for controlling the expression of a network of cytoprotective genes, including antioxidant and anti-inflammatory genes as well as genes involved in mitochondrial biogenesis. Using biochemical techniques in addition to live cell imaging and respirometry we now show that the Keap1-Nrf2 pathway is further involved in the control of mitochondrial metabolism. Experiments in mouse embryonic fibroblasts from wild-type, Nrf2 and Keap1 knockout (KO) animals show that, compared to wild-type, cells lacking Nrf2 have decreased mitochondrial membrane potential while Keap1 KO cells on the other hand show an increase. Interestingly, Nrf2 KO cells show significant inhibition of the rate of oxygen consumption, suggesting that the decrease in mitochondrial membrane potential is not due to mitochondrial uncoupling. We further demonstrate in primary neuroglia cultures (isolated from wild-type, Nrf2 KO and Keap1 knockdown mice) that this pathway has a significant effect on the FAD and NADH redox states of the cells and the defect could not be reversed by application of mitochondrial substrates. Finally, Nrf2 KO cells show increased dependence on glycolysis, as seen by both live cell imaging and western blot. In conclusion, the Keap1-Nrf2 pathway is not only important in the anti-oxidant defence of the cells but also plays a major role in energy metabolism.

**2912-Pos Board B682**
**A Surface Plasmon Resonance-Based Two-Dimensional Screen for Protein Kinase Substrates Identifies Fumarase as AMPK Target**
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This study describes a novel in vitro two-dimensional screening approach for kinase substrates that combines a biophysical interaction assay based on surface plasmon resonance and a standard in vitro phosphorylation assay. The interaction assay was optimized with appropriate prepurification procedures on a Biacore instrument. It selects for substrates interacting with a specific kinase, and can thus identify substrates that are preferentially phosphorylated e.g. by a specific kinase isoform. This approach was applied to isoforms of the heterotrimeric AMP-activated protein kinase (AMPK). AMPK is an emerging central cellular signaling hub in energy homeostasis and proliferation, but its signaling network is still incompletely understood. Using soluble rat liver proteins and full-length AMPK $\alpha 2$-$\beta 2$-$\gamma 1$ complex, several putative AMPK substrates were identified by mass spectrometry. One of them, fumarate hydratase (fumarase), was confirmed as an in vitro AMPK target which preferentially interacted with and was phosphorylated by the AMPK$\alpha 2$ isoform as shown by yeast-two-hybrid and in vitro phosphorylation assays. AMPK-mediated phosphorylation of fumarate hydratase led to significant activation of enzymatic activity in vitro and in vivo, suggesting that it is a bona fide AMPK substrate. This may have different physiological consequences, since the enzyme has a dual localization in the mitochondrial matrix and the cytosol. [AK and CP contributed equally to this work]

## Electron & Proton Transfer

**2913-Pos Board B683**
**Negative Cooperativity in the Reduction of Excitonically Interacting B-Hemes of the Cytochrome B6F Complex**
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Cytochrome b6f and bc1 (bc) complexes, which provide the central charge transfer complex in respiratory and photosynthetic electron transport chains, are symmetric dimeric structures.1 A trans-membrane electron transfer pathway between hemes bp and bn2 exists in each monomer. Based on inter-heme distances this intra-monomer pathway (bp-bn) is preferred over an inter-monomer cross-over pathway between hemes bp1. Cross-over has been reported,3,4 although the branching ratio is not known. Previous studies on ferredoxin-dependent reduction of b6f by NADPH showed only half of the b-heme reduced in thylakoid membranes,5 and biphasic reduction with isolated complex.6 It was inferred that the rapid reduction occurred through intra-monomer electron transfer and that the split circular dichroism spectrum7,8 (node of the CD spectrum in the Soret band coincides with the heme absorbance maximum), implies that the hemes bp and bn interact excitonically in the monomeric unit. Here, monophasic chemical (dithionite) reduction of monomeric b6f complex, characterized by size-exclusion chromatography, proceeds 10-20 times more rapidly than in the dimeric complex. The reduced monomer showed full amplitude of the Soret band split CD spectrum. These measurements are consistent with ''half-sites reactivity'' observed for b-heme reduction in the yeast bc1 complex,9,10 in which reduction of one monomer is associated with a reduced rate of electron transfer to the second monomer of the bc1 dimer. The mechanism for this negative cooperativity could arise from a larger reorganization energy11 for inter-monomer electron transfer, or constraints imposed by the Rieske protein in the dimer. NIH-GM38323.1Cramer et al. 2011; 2Yamashita et al. 2007; 3Lanciano et al. 2010; 4Swierczek et al. 2010; 5Furbacher et al. 1989; 6Hasan et al., 2011; 7Palmer and Degli-Esposti 1994; 8Schoepp et al. 2000; 9Covian and Trumpower, 2008; 10Castellani et al. 2010; 11Marcus and Sutin 1985.

**2914-Pos Board B684**
**Energy Transduction by Bacterial Complexes I**
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Complex I (CpI) of respiratory chains is an energy transducing enzyme present in most bacteria and in all mitochondria. It is still the least understood complex of these chains, in spite of the structural data recently available[1-3]. This complex catalyses NADH:quinone oxidoreduction, coupled to ion translocation across the membrane.